

# Detection of Aminoglycoside, Sulfonamide and Tetracycline resistance genes in *Escherichia coli* isolated from bovine milk samples

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**Abstract:** Many studies report that the amount of antibiotic resistant bacteria has increased in recent years. The aim of this study was to evaluate the frequency of aminoglycoside (*strA*, *strB*) sulfonamide (*sulI*, *sulII*) and tetracycline (*tetA*, *tetB*) resistance genes in *E.coli* isolated from milk samples of dairy cows in the Vysočina Region. A total of 72 samples were obtained from dairy farms. Isolates were tested for the presence of afore mentioned genes by using PCR. *StrA* and *strB* were detected most frequently. In 28 samples there were no resistance genes detected, while in 7 isolates all six were identified. The remaining samples showed a varying number of resistance genes and combinations.

**Key-words:** PCR, *E.coli*, antibiotic resistance, aminoglycoside, sulfonamide, tetracycline.

## Introduction

*Escherichia coli* is one of the most common pathogens causing contagious and environmental mastitis on dairy farms [1]. Antimicrobial therapy is frequently used for treatment and prevention of this disease [2]. Wide usage of antibiotics in veterinary medicine plays a significant role in the increase of resistance [3]. As a consequence, the treatment of diseases caused by bacterial pathogens has become very difficult.

Detection of multiple-antibiotic-resistant *E.coli* strains has been reported in several studies [4, 5]. Resistances to aminoglycosides, sulfonamides and tetracyclines among *E.coli* isolates are the most prevalent [6]. The genes most often responsible for mentioned resistances are *strA* and *strB*, *sulI* and *sulII*, *tetA* and *tetB* respectively [2, 3, 6].

Many genes encoding antimicrobial resistance are located on plasmids and/or transposons [5]. This enables their transfer between and within bacterial species. Therefore, the assessment and surveillance of resistances at the genetic level is very important.

The aim of this study was to assess aminoglycoside, sulfonamide and tetracycline resistance in *E.coli* isolated from bovine milk in the Vysočina region at the genetic level.

## Material and Methods

A total of 72 *E.coli* isolates obtained from raw cow milk were used in this study. Samples were collected from dairy farms in the Vysočina Region in the Czech Republic. Total DNA extraction from milk samples and bacterial species identification were provided by using “Thermo Scientific PathoProof™ Mastitis Complete-12 assay”. For detection of aminoglycoside (*strA* and *strB*), sulfonamide (*sulI* and *sulII*) and tetracycline (*tetA* and *tetB*) resistance genes the PCR method was used. The primers (Table 1) were designed using Primer3 and verified by Oligo4 and Primer Express® Software v2.0. The reaction mixture (total volume 10 µl) consisted of 5 µl of PPP Master Mix (Top-Bio, Prague), 0.5 µl of each primer (stock concentration 10 µM), 3.5 µl of PCR water and 0.5 µl of the template DNA (20ng/µl).

PCR was performed under the following conditions: initial denaturation at 95°C for 2 min, 30 cycles of denaturation at 95°C for 30 s, primer annealing at 60°C for 30 s and elongation at 72 °C and final elongation at 72°C for 7 min. Reaction products were detected by electrophoresis using a 2% agarose gel and visualized with ethidium bromide.

Table 1 PCR primers used for detection of antibiotic resistance genes

Gene	Primer name	Primer sequence	Fragment size (bp)	Annealing temp. (C°)	Reference GenBank
<i>strA</i>	StrA-F StrA-R	TACCGGACGAGGACAAGAGT GACCCGTGCATTGAAGAGTT	165	60	NC_001740
<i>strB</i>	StrB-F StrB-R	GGCGATTATAGCCGATCAAA TCAGCCGGATCGTAGAACAT	174	60	NC_001740
<i>sulI</i>	SulI-F SulI-R	GACGAGATTGTGCGGTTCTT CCGACTTCAGCTTTTGAAGG	160	60	X12869
<i>sulIII</i>	SulIII-F SulIII-R	TGGTGTGGCCTATCTCAATG CGCAATGTGATCCATGATGT	160	60	M36657
<i>tetA</i>	TetA-F TetA-R	TGTCCGACAAGTTGCATGAT CCTTGAACGGCCTCAATTT	178	60	X00006
<i>tetB</i>	TetB-F TetB-R	GCCAGTCTTGCCAACGTTAT CGATGCGCCTATTAATGACA	178	60	J01830

## Results and Discussion

Aminoglycoside resistance genes were detected most often. In 35 isolates (48.6%) the *strA* resistance gene was detected, while 42 (58.3%) contained the *strB* gene. Similar results were obtained by Schweiger et al. [3] where *strA* was found in 52.6% porcine and 61.2% human origin samples. The occurrence of *strB* was 54.7% in porcine and 63.8% in human origin samples. This contrasts with Lanz et al. [7], who found both genes in only 4.7% of animal isolates and Srinivason et al. [2], who detected both genes in 8.5% of dairy samples.

Genes responsible for sulfonamide resistance were detected less often: *sulI* in 27 (37.5%) and *sulIII* in 22 (30.6%) samples. Some research groups report a significant prevalence of *sulIII* over *sulI* in *E.coli* samples, e.g. Sunde and Norström [8] found *sulIII* in 75% of the meat samples and *sulI* only in 16%. Karczmarczyk et al. [9] detected above mentioned genes in 90% and 26% of the environmental samples collected at a cattle farm, respectively. In contrast, Maynard et al. [5] found *sulI* in 79% and in 36% of *E. coli* isolates obtained from pigs. On the other hand Dolejska et al. [10] and Drugdova and Kmet [11] report about low prevalence of both genes in avian *E.coli* isolates.

Genes responsible for tetracycline resistance were detected least frequently. *TetA* was detected in 14 (19.4%) and *tetB* – in 21 (28.2%) of the isolates. Bryan et al. [12] also observed a prevalence of *tetB*, but at a higher percentage (35% and 63% respectively) in diverse human and animal sources. Mayhard et al. [5] identified

a significant prevalence of *TetB* over *TetA* (80% and 25%). This contrasts with Karczmarczyk et al. [9] who reported *tetA* being present twice as often in *E.coli* isolates than *tetB*. Lanz et al. [7], Sunde and Norström [8] and Koo and Woo (2011) also detected *tetA* more frequently. Srinivasan et al. [2] found *TetA* only in 10.9% of their samples and have not detected *TetB*.

In total, 8 *E.coli* isolates carried five antibiotic resistance genes, 10 carried four genes, 4 carried three genes 12 carried two genes and 3 carried one gene, in different combinations. In 7 samples all six antibiotic resistance genes were detected. There were no sought genes identified in 28 *E.coli* isolates.

## Conclusion

This study reported about prevalence of aminoglycoside (*strA*, *strB*) sulfonamide (*SulI*, *SulIII*) and tetracycline (*TetA*, *TetB*) resistance genes in *E.coli* isolated from milk samples of dairy cows in the Vysočina Region.

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